Role of *Gardnerella vaginalis* in the Pathogenesis of Bacterial Vaginosis: A Conceptual Model

Jane R. Schwebke,¹ Christina A. Muzny,¹ and William E. Josey²

¹Department of Medicine, University of Alabama at Birmingham, and ²Department of Gynecology and Obstetrics, Emory University, Atlanta, Georgia

**Background.** Bacterial vaginosis (BV) is the most common cause of vaginal discharge and is associated with important public health complications such as preterm birth and acquisition or transmission of human immunodeficiency virus and sexually transmitted infections. Continued controversy concerning the pathogenesis of BV has led to a lack of progress in prevention and management of this infection.

**Methods.** Development of a conceptual model for the pathogenesis of BV based on review of past and current research.

**Results.** Our model suggests that BV is initiated by the sexual transmission of *Gardnerella vaginalis*, which has the appropriate virulence factors to adhere to host epithelium, create a biofilm community, and successfully compete with lactobacilli for dominance in the vaginal environment. The genetic diversity of *G. vaginalis* may result in virulent and avirulent strains. Symbiotic relationships with normally dormant vaginal anaerobes lead to increases in the latter which contribute to the symptoms of BV.

**Conclusions.** *G. vaginalis* is the pathogen responsible for the initiation of BV. Future research should focus on preventing its transmission and improved therapeutics for the biofilm infection that is caused by this pathogen and host anaerobes.

**Keywords.** bacterial vaginosis; biofilm; Gardnerella; vaginal flora; lactobacilli.

We are prepared to present evidence that the vast majority of so-called “non-specific” bacterial vaginitides constitute a specific infectious entity caused by a single etiological agent... We have assigned the name *Haemophilus vaginalis* to this newly isolated bacillus. Gardner and Dukes, 1955 [1].

Bacterial vaginosis (BV) is the most prevalent cause of symptomatic vaginal discharge and is associated with complications of reproductive health, such as preterm birth and acquisition or transmission of sexually transmitted infections, including human immunodeficiency virus infection (STI) [2, 3]. Control of BV has been advocated for decreasing the prevalence of these complications, but the precise etiology of BV remains unknown. As a result, current treatment regimens and prevention strategies are inadequate. Such a lack of understanding not only inhibits our ability to effectively manage BV but also severely affects our ability to prevent its associated complications. It is clear that BV is characterized by dramatic changes in the vaginal flora, from lactobacillus predominance to a marked decrease in lactobacilli, particularly those producing hydrogen peroxide [4]. Lactobacilli are replaced by the facultative anaerobe *Gardnerella vaginalis*, and strictly anaerobic bacteria markedly increase in concentration [5]. As reviewed elsewhere [6, 7], the epidemiology of BV strongly indicates that it is acquired via sexual transmission. Reviewing the literature, past and present, we have developed a conceptual model for the pathogenesis of BV (Figure 1).

**CONCEPTUAL MODEL OF THE PATHOGENESIS OF BV**

**Development of the Vaginal Microbiome**

The vaginal flora is acquired shortly after birth from maternal and environmental sources [8]. In a study of
prepubertal girls, Hill et al [9] demonstrated the presence of anaerobic organisms in the vaginal fluid in the majority of girls yet were unable to demonstrate the presence of *G. vaginalis* by culture, even though it was specifically sought. At puberty, with the production of estrogen, the vaginal flora changes to lactobacillus predominance. Estrogen promotes the deposition of glycogen in the vaginal epithelium, which in turn is used as a food source by the saccharolytic lactobacilli. The subsequent creation of lactic acid as their metabolic end product lowers the vaginal pH to <4.5 [10]. In addition, growth of lactobacilli increases the reduction-oxidation (redox) potential of the vagina, thus inhibiting the growth of the indigenous anaerobes [11].

**G. vaginalis in the Female Genital Tract**

Epidemiologic data strongly support the sexual transmission of *G. vaginalis* [6]. *G. vaginalis* has been recovered from vaginal...
fluid in close to 100% of women with clinically diagnosed BV [13]. Other frequently identified BV-associated bacteria, recovered at variable rates in the vaginal microbiome, are the genital mycoplasmas and various strict anaerobes including species of BVAB1, BVAB2, BVAB3, Atopobium, Leptotrichia, Megaplasma, Prevotella, and Dialister [14]. However, the assortment of strict anaerobes found in individuals with BV is heterogeneous [15].

Several published studies have demonstrated the detection of G. vaginalis from women who did not meet the clinical criteria for BV [16, 17]. These studies, however, were not rigorous in their definition of normal or optimal flora and failed to take into account that there are women who meet neither the clinical definition of BV nor the definition of normal vaginal flora, that is, women with intermediate flora by Nugent score (scores of 4–6) [18]. Teixeira et al [19] found G. vaginalis in only 17.6% of “healthy” women without a clinical diagnosis of BV, but there was no documentation that these women had none of the Amsel criteria or an optimal Nugent score of 0–3. Similarly, Burton et al [20] reported G. vaginalis to be present in only 19% of women without BV but provided no Nugent score. The finding of G. vaginalis in these women without clinical evidence of BV or even possibly intermediate flora likely represents asymptomatic infection as is seen in all STDs. The specific host-pathogen interactions which result in limiting the virulence of the colonizing pathogen are poorly understood. However, if G. vaginalis were a part of the normal vaginal flora, one would expect it to be present in all women and in prepubertal girls as well [9].

**G. vaginalis in the Male Genital Tract**

In males, G. vaginalis has been recovered from the urethra and from seminal fluid in several studies [1, 21, 22]. The presence of BV-related microorganisms in the male genital tract suggests a possible reservoir and supports the theory of sexual transmission of BV. It has been hypothesized that semen, because of its alkaline properties, may alter the acidic pH of the vagina and lead to BV [23]. However, sexual transmission of BV without exposure to semen in a heterosexual couple strongly suggests that it is not the semen itself that contributes to the development of BV but rather microbes transmitted via sexual activity [24]. By analogy, this is supported by the apparent sexual transmission of BV among women who have sex with women (WSW) only [25]. Using sophisticated sequence variation techniques, Eren et al [26] were able to show that sexual partners shared the same strains of G. vaginalis. Insight into the site of colonization or infection of G. vaginalis in males is provided by a study of the microbiome of adolescent boys. Nelson et al [27] found G. vaginalis in 28% of urine samples but failed to detect G. vaginalis from samples of the coronal sulcus. In females, BV is an infection of the squamous epithelium as opposed to the columnar epithelium of the cervix. Thus, it is plausible that in males, colonization and infection of the genital tract is restricted to the distal urethra, which is lined with squamous epithelium. Holst [22] showed that BV-related organisms, including G. vaginalis, seemed to transiently colonize male partners of women with BV. As with Trichomonas vaginalis in men, another pathogen that infects the squamous epithelium of the vagina, there is probably a high spontaneous resolution rate owing to the inhospitable environment of the distal urethra in males [28]. BV may be more common among WSW than in heterosexual women [29]. Sexual exchange of infected vaginal fluid may be a more efficient mechanism for the transmission of BV between WSW than behaviors that occur during heterosexual sex, thus accounting for the higher prevalence of BV in WSW.

**Adherence of G. vaginalis to Host Epithelium: Initial Steps in Invasion**

Examination of vaginal biopsy specimens demonstrates that BV is a biofilm community adherent to the vaginal epithelium and that G. vaginalis is the predominant component of the biofilm mass [12]. The initial steps of establishing infection include adherence to host receptor sites, production of cytotoxic substances specific for host cells, and biofilm formation. In the case of G. vaginalis, production of vaginolysin, a cholesterol-dependent cytolysin, is species specific for human cells and encodes a pore-forming toxin that binds to the CD59 human complement regulatory molecule [30]. This cytotoxin assists in the initial adherence of G. vaginalis to the host epithelial cells [30]. Investigators have recently examined virulence factors of G. vaginalis compared with other BV-associated bacteria. Patterson et al [31] examined adherence, biofilm formation, and cytotoxicity in vitro for G. vaginalis strains isolated from women with BV as well as other BV-associated bacteria, including Atopobium, Prevotella, and Mobiluncus. They found that among these organisms only G. vaginalis demonstrated all 3 virulence factors and suggested that the other organisms may be relatively avirulent opportunists that colonize after initiation of infection by G. vaginalis. Recent work by Machado et al [32] found that in vitro, among BV-associated bacteria, G. vaginalis had the greatest capacity to adhere to epithelial cells in the presence of Lactobacillus crispatus.

**G. vaginalis Mechanisms of Infection**

Subsequent to initial adherence to the host cell, the invading bacteria multiply and may produce a biofilm community as a means of future survival [33]. Production of biofilm is critical to the survival of G. vaginalis in the vagina. Sialidase, which is produced by some strains of G. vaginalis, may enhance production of biofilm through its mucinase activity [34]. It has been shown in vitro that biofilms of G. vaginalis are far more tolerant of lactic acid and hydrogen peroxide produced by lactobacilli than are planktonic forms of G. vaginalis [35]. Once this critical mass of bacteria is present, quorum sensing or bacterial
“cross-talk” occurs, allowing the bacteria to effectively manage their new community [36]. At the same time, it is necessary for the invading pathogens to engage in “chemical warfare” with native species. In the case of BV, the native species is composed of lactobacilli. It has been shown that certain species of Lactobacillus are capable of producing inhibitory compounds, or bacteriocins, which are active against bacilli [37]. Conversely, it has been shown that G. vaginalis produces bacteriocins active in vitro against lactobacilli [38]. G. vaginalis has also been shown to demonstrate antibiosis (antagonistic interactions) via other undetermined mechanisms [39]. In 1991, Nagy et al [39] published their work on antibiosis among vaginal organisms and concluded that “it is probable that the growth inhibition of lactobacilli caused by certain strains of G. vaginalis is one of the first steps in the change in flora characteristic of BV.” Evidence for competition between lactobacilli and G. vaginalis can be demonstrated from daily vaginal Gram stains of women with intermediate vaginal flora (Figure 2) [40]. In these women, lactobacilli and G. vaginalis vary as the dominant organism throughout the menstrual cycle. G. vaginalis dominates at the time of menses, suggesting competition for iron as a substrate for the bacteria [41]. It is known that heme favors the growth of proteolytic organisms, such as G. vaginalis [42]. It is highly likely that these observed fluctuations in the levels of lactobacilli and G. vaginalis represent competition for resources between lactobacilli and G. vaginalis.

Syndrome of BV-Symbiosis in the Vaginal Microbiome

G. vaginalis, as a facultative anaerobe, may be able to tolerate the high oxidation-reduction (redox potential) of a healthy vaginal microbiome, unlike strict anaerobes [11]. Similar to facultative anaerobes involved in the initiation of oral diseases, it is likely that G. vaginalis begins the process of creating a lower redox potential, which is then suitable for the overgrowth of strict anaerobes, normally present in very low numbers [42]. In addition, G. vaginalis is a proteolytic bacterium that produces amino acids through its metabolism. It has been shown that Prevotella bivia, a strict anaerobe, uses amino acids as its fuel source and as a result produces ammonia, which in turn is used by G. vaginalis [43]. Therefore, a symbiotic relationship between G. vaginalis and strict anaerobes is another mechanism whereby G. vaginalis enhances the growth of anaerobes in the vagina. Moreover, this symbiotic relationship with the production of ammonia would cause a shift to a more alkaline pH, which is inhospitable to lactobacilli [39]. In vitro work has shown that the addition of anaerobes to a G. vaginalis biofilm enhances the growth of G. vaginalis [32]. These symbiotic relationships are responsible for the microbiological findings that define BV as well as the typical vaginal signs (sloughing of the vaginal epithelium visualized as “clue cells” and amine odor resulting from metabolic by-products of the increased numbers of BV-associated anaerobes [44]).

Genetic Diversity in G. vaginalis

Genomic sequencing has recently shown differences in virulence factors among strains of G. vaginalis [45]. Harwich et al [46] examined virulence factors for a strain of G. vaginalis from a woman with BV and another without BV. They found impaired adherence in the non-BV isolate and suggested that there may be both commensal and pathogenic strains of G. vaginalis. However, as in other studies, there is no mention of the Amsel or Gram stain characteristics of the woman without BV. Furthermore, this type of work needs to be replicated with multiple isolates. Recent comparative genomic analyses of 17 clinical isolates of G. vaginalis suggested that the species can be subdivided into 4 clades or even that there may be multiple species of G. vaginalis. Ahmed et al [47] found that the degree of diversity among the strains was exceptionally high for a single species.

Koch’s Postulates Fulfilled

Koch’s postulates of infection, developed in 1884, state that to prove causation between a microbe and a disease the following conditions must be met: (1) the microorganism must be found in abundance in all organisms suffering from disease and not present in those without the disease; (2) the microorganism must be isolated from a diseased organism and grown in pure culture; (3) the cultured microorganism should cause disease when introduced into a healthy organism; and (4) the microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent. In the case of G. vaginalis as the causative agent for BV, these postulates have been fulfilled. G. vaginalis is found in nearly 100% of cases of BV, and it is isolated as the predominant bacteria from women with BV along with small amounts of indigenous vaginal flora [13]. Moreover,
Criswell et al [48] caused clinical BV in healthy women who had negative cultures for G. vaginalis and no clinical signs of BV before vaginal inoculation with a pure culture of G. vaginalis, in its logarithmic phase. In addition they reisolated G. vaginalis from these women after initiation of BV. Although G. vaginalis has been isolated from women not meeting the Amsel criteria for BV, this does not mean that these women are without disease. Furthermore, in many instances the causative organism is present in the host but not causing overt disease such as asymptomatic carriage of STIs and gastrointestinal pathogens such as Salmonella typhi [49].

CONCLUSION

BV is an important public health issue, yet its pathogenesis remains controversial. Its epidemiology strongly favors the hypothesis that it is an sexually transmitted infection. Reviewing the available data from the past 50 years, we have developed a model for BV pathogenesis which is similar in all respects to any human infection. A significant body of data supports the pathogenicity of G. vaginalis. Studies have confirmed that it is the dominant component of the BV biofilm, strongly suggesting that other bacteria in the biofilm are opportunistic secondary intruders. The presence of virulence factors in G. vaginalis, shown to be present 30 years ago, has been recently confirmed by several studies. Prevention and treatment strategies specifically for G. vaginalis should be tested to determine their efficacy in decreasing rates of BV and its complications.

Notes

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